

An Analytical Approach for Insulin-like Growth Factor Receptor 1 and Mammalian Target of Rapamycin Blockades in Ewing Sarcoma

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Abstract

We present preliminary results that quantify network robustness and fragility of Ewing sarcoma (ES), a rare pediatric bone cancer that often exhibits de novo or acquired drug resistance. By identifying novel proteins or pathways susceptible to drug targeting, this formalized approach promises to improve preclinical drug development and may lead to better treatment outcomes. Toward that end, our network modeling focused upon the IGF-1R-PI3K-Akt-mTOR pathway, which is of proven importance in ES. The clinical response and proteomic networks of drug-sensitive parental cell lines and their drug-resistant counterparts were assessed using two small molecule inhibitors for IGF-1R (OSI-906 and NVP-ADW-742) and an mTOR inhibitor (mTORi), MK8669, such that protein-to-protein expression networks could be generated for each group. For the first time, mathematical modeling proves that drug resistant ES samples exhibit higher degrees of overall network robustness (e.g., the ability of a system to withstand random perturbations to its network configuration) to that of their untreated or short-term (72-hour) treated samples. This was done by leveraging previous work, which suggests that Ricci curvature, a key geometric feature of a given network, is positively correlated to increased network robustness. More importantly, given that Ricci curvature is a local property of the system, it is capable of resolving pathway fragility. In this note, we offer some encouraging yet limited insights in terms of system-level robustness of ES and lay the foundation for scope of future work in which a complete study will be conducted.

1. Introduction

Quantifying the fragility and robustness of biological cell signaling pathways is a problem of paramount importance in guided targeted cancer therapy. In this note, we restrict our attention to Ewing sarcoma - which like many cancer types - often circumvents the effect of drug therapies due to counter-regulatory signaling cascades. In ES, compelling evidence has shown that the insulin-like growth factor 1 receptor (IGF-1R) is particularly important [1, 2, 3, 4] as it is involved in the activation of multiple oncogenic pathways including the downstream activation of phosphoinositide-3-kinase/Akt/mammalian target of rapamycin (mTOR) [5]. To this end, studies have shown that targeting IGF-1R can inhibit growth in ES cells in vitro, in xenograft models, and in 10-14% of ES patients who had dramatic (albeit short-lived) clinical responses to IGF-1R Abs in clinical trials conducted since 2007 [6, 7]. As such, combinatorial inhibition of IGF-1R and mTOR has been shown to triple the response rate as compared to sole IGF-1R inhibition [8, 9, 10, 11]. Although drug-resistant ES samples still arise, this noted synergistic effect

with respect to duration of response lends support to notions of network “fragility” and “robustness” [12]. That is, on the one hand, tumors can initially be quite fragile to dual IGF-1R/mTOR targeting while, on the other hand, quickly acquire drug resistance, that manifests as robustness mediated by alternative feedback loops that promote cell survival. This preliminary note lays the continued foundation to the follow key question:

Central Question: *Given the complexity and cost of selecting drug candidates, can we quantify (and therefore predict) pathway fragility in order to uncover a set of a set of n -tuple targets that can properly disrupt the alternative signaling cascades attributed to ES tumor growth?*

In addressing this critical question, a host of new “-omics” technologies has provided cancer biologists with unparalleled access to expression data across the proteome or transcriptome that allows scientific exploration of tumor phenotype at the network level, rather than a reductionist approach confined to specific protein-protein interactions. As such, one can consider cellular interactions apart of the interactome as a complex dynamical system represented by weighted graphs [13, 14, 15, 16]. In doing so, we are able to compute certain geometric features in hopes of elucidating certain key attributes and stylized facts of the underlying system. One such property is the geometric notion of curvature [17]. We have previously shown that increased Ricci curvature is positively correlated with increased robustness, herein expressed as $\Delta Ric \times \Delta R \geq 0$ [18]. Generally speaking, robustness relates to the rate at which a given dynamical system returns to its original (normal) state following a perturbation or external disturbance. Though essential for maintaining homeostasis in living organisms, as applied to cancer, robustness can be interpreted as the ability of malignant cells to coopt or reshape signaling pathways in a way that preserves cell survival despite perturbations induced by drug therapy. In practice, we note that malignant cells may acquire robustness against some perturbations, and in so doing, unearth other areas of fragility that would not otherwise have existed. This oncogene addiction can be exploited using biologically targeted therapies that selectively kill cancer cells and belies the use of many of today's most effective oncology drugs.

We refer the reader to [12, 20, 21, 22] for a precise definition of robustness and several works [23, 24, 25, 26, 27] regarding the details of curvature. Nevertheless, the key concept here is that on a given graph/interactome, we are able to employ Ricci curvature to express system-level and pathway fragility. In the context of clinical success, it has been argued that feedback loops are essential to the function of ES with previous rationale for the success of joint IGF-1R/mTOR inhibition [5]. Stated in the context of graph theory, this feedback or the number of triangles in an interactome (redundant pathways) can be characterized by a lower bound of Ricci curvature [18, 29, 30]. We show some key pathways connected to IGF-R1 in Figure 1.

We previously demonstrated that cancer networks exhibit a higher degree of global robustness (in terms of network curvature) in relation to their normal counterparts [18]. We now focus our attention on comparing robustness for drug resistant, drug sensitive, 72-hour treated samples specifically in ES (treated by OSI-906/NVP-ADW-742 and MK8669) by constructing protein-to-protein correlation expression networks derived from a selected reverse phase protein assay (RPPA) panel for each associative group. We note that the protein interaction topology may change under selective pressure of chemotherapy, forging new protein-protein interactions that typically would not exist physiologically. For simplicity, we fix the topology of the networks using prior data on known physical interactions allowing only the weights to evolve. Then, by treating each network as a random walk (e.g., the path traversed by a molecule as it travels in a liquid), we attempt to exploit the underlying dynamics of specific protein-to-proteins interactions. Nevertheless, we caution readers on the current preliminary results due to the limitation of sample size and scope of network pathways - ***this work is presented with the forethought that a larger exploratory analysis will be conducted to ensure clinical benefit.***

The remainder of the present note is outlined as follows: In the next section, we revisit a previously employed method for measuring network and pathway fragility via Ricci curvature, in the Ollivier sense [28, 27]. Section 3 presents preliminary results demonstrating that resistant ES cell samples are more robust than their untreated counterparts with drug sensitive samples being the most fragile; this is illustrated in Figure 2. We also elucidate

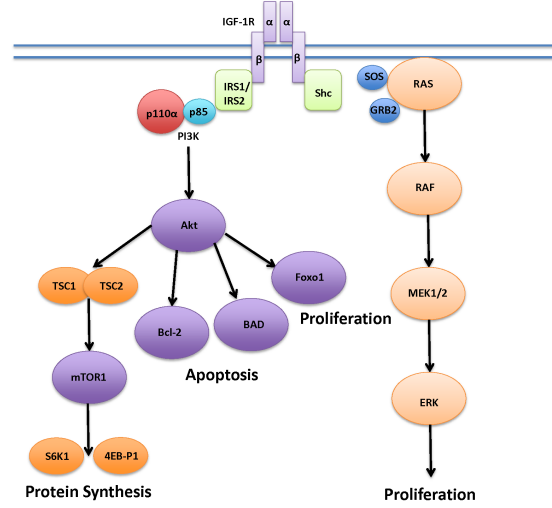


Figure 1. A simple schema of the IGF-1R pathway. IGF-1R is activated through binding of IGF-1 and IGF-2. Autophosphorylation and crosslinking of IGF-1R leads to activation of IRS-1 and Shc and from there downstream activation of PI3K/Akt and Ras pathways.

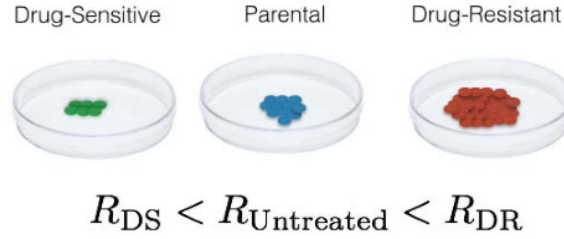


Figure 2. Proposed behavior of ES cells to IGF-1R and mTOR blockades.

possible pathways (e.g., MEK1, IRS1, PI3K) that have been suggested as possible significance with regards to contribution to the robustness in ES. We conclude with a discussion of future work.

2. Measuring Fragility via Ricci Curvature

As mentioned in the previous section, we utilize the stylized fact that increases in Ricci curvature are positively correlated to increases in robustness, i.e., $\Delta Ric \times \Delta R \geq 0$. Given that our work is confined to a discrete space of an interactome/graph in which the nodes on a graph represent a particular protein/gene and edges represent an direct interaction, we employ a notion of a Ricci curvature [27] inspired through coarse geometry. That is, in order to generalize Ricci curvature to a discrete space, certain properties of curvature are adopted from the continuous setting. In this case, the intuition is that the transportation distance between two small (geodesic balls) is less than the distance of their centers on a positively curved space, greater than distance of their centers on a negatively curved space, and equal to the distance on a space of zero curvature. Specifically, if we let (X, d) be a metric space equipped with a family of probability measures $\{\mu_x : x \in X\}$, we define the *Ollivier-Ricci curvature* $\kappa(x, y)$ along the geodesic connecting nodes x and y via

$$W_1(\mu_x, \mu_y) = (1 - \kappa(x, y))d(x, y), \quad (1)$$

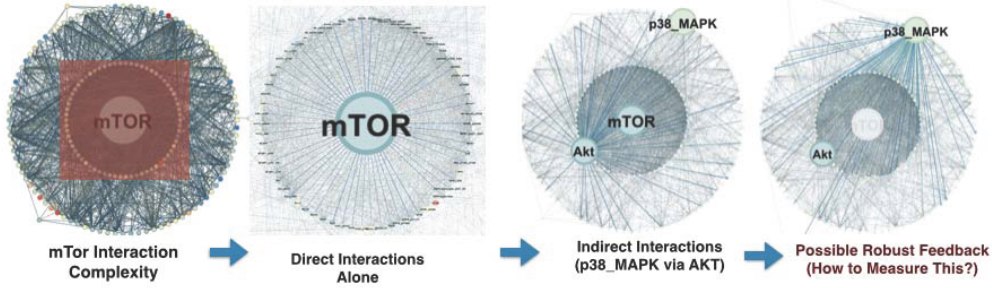


Figure 3. Modeling the selection for a drug target candidate. An example of the complexity of choosing drug targets. Only focusing on the mTOR signaling pathways, there exists a vast set direct interactions compounded further by the influence of indirect interaction (e.g., mTOR to p38.MAPK via AKT). The resulting structures builds various feedback (not seen in this figure) circumventing the effect of a particular therapy (e.g., series of interactions leading p38.MAPK back to mTOR) . The methodology proposed in this note attempts to provide a solution that can quantify pathway fragility and robustness of such indirect interactions. In turn, we can then begin to understand mechanisms of resistance

where W_1 denotes the Earth Mover's Distance (Wasserstein 1-metric) [31, 32] and d is the geodesic distance on the graph. For the case of weighted graphs, we set

$$d_x = \sum_y w_{xy} \quad (2)$$

$$\mu_x(y) := \frac{w_{xy}}{d_x}, \quad (3)$$

where d_x is the sum taken over all neighbors of node x and where w_{xy} denotes the weight of an edge connecting node x and node y ($w_{xy} = 0$ if there is no direct edge connecting node x to node y). The measure μ_x may be regarded as the distribution of a one-step random walk starting from x , with the weight w_{xy} quantifying the strength of interaction between nodal components or the diffusivity across the corresponding link (edge). Computationally, the above term W_1 may be computed as a linear program [33] allowing for several numerical advantages [18]. From this, we can then measure the fragility of a given (not necessarily adjacent) pathway through decreases/increases in the term $\kappa(x, y)$ with the hopes that such measures will be able to guide targeted therapies through such biological complexity. An example of this complexity can be seen in Figure 3.

3. Results

In this section, we test this hypothesis using proteomic expression data taken from ES cells cultured in vitro and present the resulting computation for network curvature/robustness related to IGF-1R and mTOR blockades in ES cell lines.

3.1. Data and Network Construction

The preliminary data was obtained using protein expression data, measured via highly sensitive reverse-RPPA in experiments conducted at MD Anderson that investigated mechanisms of resistance in TC32 and TC71 ES cell lines to IGF-1R and/or mTOR inhibition [19]. Briefly, two independent methods were used to generate drug resistance in ES cell lines grown in monolayer culture. In the first method, parental cell lines were exposed to steadily increasing drug concentrations of OSI- 906 or NVP-ADW-742 for 7 months. In the second method, ES cells were exposed to exceedingly high concentrations for 72 hours before selecting viable clones, which were expanded. These two groups were combined along a set of parental samples; herein referred to as **drug resistant ES cell lines**. The resulting drug-resistant cells were compared to untreated parental cells and to cells exposed to

IGF-1R Inhibitor	72-Hour	Untreated	Resistant
Average Curvature	0.1926	0.2300	0.2409
5 % Left Tail (Avg.)	-0.0990	-0.0542	-0.0476
3% Left Tail (Avg.)	-0.1758	-0.1179	-0.1111
1% Left Tail (Avg.)	-0.3745	-0.2777	-0.2817

Table 1. Global network results for IGF-1R treatment show that, on average, robustness as measured by Ricci curvature is higher in drug resistant samples with drug sensitive samples being the most fragile. The analysis presented in this table shows both average Ricci curvature, minimum curvature, and average value of the left tails (at specific lengths) in order to characterize the “shifts” seen within each network.

the studied drugs for 72 hours; herein referred to as **drug sensitive**. A separate, but similar experiment was repeated with MK8669 to generate a complementary data set targeting the effects of mTOR inhibition. All together, there consists a set of 12 drug resistant samples, 4 drug sensitive samples, and 8 parental (untreated) samples for the OSI-906/NVP-ADW-742 (IGF-1R) treatment while 8 drug resistant samples, 2 drug sensitive samples, and 3 parental samples for the MK8669 (mTOR blockade) treatment were obtained. Using the RPPA protein expression data (consisting of a small set of 112 and 165 proteins for IGF-1R and mTOR experiments, respectively), we were able to generate a protein-to-protein network through known physical interactions and with weights that were computed from the correlation across such samples within each group. We should note that although the OSI-906/NVP-ADW-742 targeting IGF-1R inhibition, the data examined does not include IGF-1R expression and lacks several proteins of interest (due to experimental reasons conducted outside the scope of the present work). However, the data analyzed for MK8669 treatment did include mTOR and adjacent interactions/proteins in this cell-signaling pathway. Nevertheless, six ES networks characterizing the drug resistant, drug sensitive, and untreated cases for IGF-1R and separately, mTOR inhibition, were constructed and were sufficient for macroscopic analysis.

3.2. Global Network and Pathway Fragility

Following our previous work [18], we conducted a global analysis with respect to network curvature on six networks that emerged from each of the two drugs and three treatment states to highlight the inherent robustness in ES resistant cell lines. In particular, Table 1 and Table 2 present the average Ricci curvature in each of the three groups for samples treated with NVP-ADW-742/OSI-906 and MK8669 as well as average value of the left tails (at given lengths of 5%, 3% and 1%). As such, one can see that the difference between drug resistant and untreated (and/or drug sensitive) samples is positive signifying robustness. Moreover, the drug sensitive samples that were only transiently exposed to IGF-1Ri and/or mTORi for 72 hours exhibited fragility when compared to the untreated and resistant cases, mimicking the higher state of drug resistance observed clinically. In addition to the average statistic, one can consider the left tail of the distribution as the lower bound of Ollivier-Ricci curvature as opposed to simply taking minimum value which maybe sensitive to topological errors. The motivation for analyzing the lower bound / left tails of the distribution is significant as it is intimately connected to a notion of entropy which also serves as a proxy for robustness [18, 23]. While beyond the scope of this work, entropy is measure of the number of specific ways in which a thermodynamic system may be arranged, and has been commonly understood as a measure of disorder or randomness in a given system.

While global results provide a quantitative indicator of network robustness/fragility, the clinical benefit of the above method is that the methodology measures fragility in pathways (that need not be necessarily directly connected) for particular targets. We can analyze particular targets in varying manners, one of which is through scalar

mTor Inhibitor	72-Hour	Untreated	Resistant
Average Curvature	0.0715	0.1204	0.2168
5 % Left Tail (Avg.)	0.1937	-0.1268	-0.0311
1% Left Tail (Avg)	-0.2800	-0.2066	-0.0974
1% Left Tail (Avg)	-0.4941	-0.4246	-0.2446

Table 2. Global network results for mTOR inhibition via MK8669 also shows that, on average, robustness as measured by Ricci curvature is higher in drug resistant samples with drug sensitive samples being the most fragile. The analysis presented in this table shows both average Ricci curvature, minimum curvature, and average value of the left tails (at specific lengths) in order to characterize the “shifts” seen within each network.

curvature [18]. Specifically, we define scalar curvature in this text as

$$S(x) = \sum_y \kappa(x, y) \quad (4)$$

which is simply the sum all Ricci curvature over direct and indirect pathways. The decision to employ this definition as opposed to simply summing over direct interactions is due to the fact that we would like to measure activity of a particular protein with respect to all other proteins in the context of robustness/fragility. Thus, unlike differential expression, scalar curvature defined in this manner measures *hidden effects* due to the underlying network. To this end, Table 3 and Table 4 present several different proteins and their scalar curvature under drug resistant, drug sensitive, and untreated cases.

Protein	72-Hour	Untreated	Resistant
mTor	41.5497	42.3427	42.9061
IRS1	43.3847	45.7001	49.7784
PRAS40	50.2089	46.6663	48.1019
PS706K	42.2576	50.1424	50.3668
Bax	29.1603	31.2746	28.3424
Smad3	39.2377	45.4066	49.8541

Table 3. Scalar curvature results for several possible targets in Ewing Sarcoma when samples were treated with OSI- 906 or NVP-ADW-742, which are known to inhibit IGF-1R/IR- α and IGF-1R.

Interestingly, we observe in Table 3 those direct/indirect pathways involved with particular proteins, i.e., IRS1, that are in the “neighborhood of” of IGF-1R and IR- α elicit an increase to their robustness from drug sensitive samples to drug resistant samples. On the other hand, mTOR seems, for the most part, unaffected by OSI-906/NVP-ADW-742 treatment as compared to the relative change in Table 4. This is in line with prior reports, which suggest the single-agent IGF-1R directed therapies are rarely potent enough to completely inhibit mTOR and its downstream effectors [36]. As seen in Figure 4, this direct quantitative effect from the treatment of MK8669 with regards to mTOR fragility is what one would expect when targeting a particular protein.

Moreover, several other insights are noted. In particular, treatment with MK8669 responds to an increase activation of IRS1 as opposed to OSI-906/NVP-ADW-742. This is expected, as others have reported that mTORi disturb the negative feedback loop upon IRS-1 by p70S6K [37, 38, 39, 40]. Another protein, PRAS40, apart of the mTOR complex I (mTORC1), elicited an increase in fragility when treated with MK8669. Biologically, this falls in line with previous understanding of mTORC1 sensitivity to such targeting. The opposite effects were registered in IGF-1R samples providing further (yet very limited) evidence that mTOR serves as a powerful feedback structure when solely inhibiting IGF-1R [5]. In short, while such results are very preliminary, much of

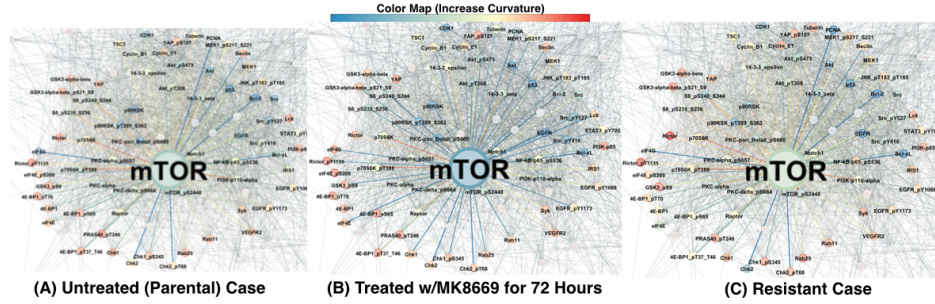


Figure 4. Quantification of network robustness and fragility in ES cells treated with mTOR inhibition. This illustrates the increase in fragility of mTOR and the surrounding “neighborhood” as a result of the treatment of MK8669 for 72-hours. Unfortunately, resistance becomes to build over time muted the the therapeutic effect of solely inhibiting mTOR.

the biological understanding seen in previous literature holds true in our analysis on curvature on this small set of samples.

4. Future Work Summary

In the above analysis, there exists several limitations that will need to be addressed. First and foremost, the sample size and RPPA panel of proteins are quite small and for any clinical benefit to arise, we must extend the above analysis. Though the proteins selected within the RPPA panel represent only a small fraction of the entire proteome, they are enriched for proteins linked to cancer (either oncogenes or tumor suppressors). This is both a weakness and strength; while the networks are biased toward cancer-related pathways, enrichment ensures the most important oncogenic pathways are comprehensively assessed. Second, though *in silico* computational analysis is cheap, preclinical drug testing platforms can be expensive to perform and replicate. A third limitation, reported by our group and others, is that ES samples cultured within traditional monolayer surfaces are prone to phenotypic drift that, over time, allows cells grown in long-term culture to diverge from the phenotype that naturally exists in human tumors. Though the present study used standard tissue culture methods, future experiments may benefit from the use of biomimetic three-dimensional (3D) tissue engineered ES tumors, pioneered by our laboratory, that reliably maintains several key hallmarks of cancer (proliferation rate, morphology, etc.) [34, 35, 19]. Despite these challenges, our results suggest a path forward that yields a powerful quantitative framework that may be used to concretely measure whether therapies are exerting their intended destabilizing effect upon cancer survival. Notably, while our focus was narrowly tailored toward the IGF-1R/mTOR pathways base upon its unquestioned importance in ES, we have only tapped the surface of what is possible using our mathematical model, and other pathways are likely to prove equally or more important. As we address additional and heretofore-unexplored pathways in the context of robustness and fragility, we demonstrated that drug resistant samples are more robust than untreated or drug sensitive samples. While this finding is intuitive, an accurate calculation of robustness, as enabled by employing the concept of Ricci curvature to biological systems, may provide new clues that help pinpoint an optimal approach to prevent or defeat drug-resistant ES.

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Protein	72-Hour	Untreated	Resistant
mTor	45.5299	54.6202	65.5548
mTor_pS2448	49.5302	54.0729	64.7180
IRS1	59.6806	58.5469	72.8878
PRAS40	64.7558	71.7083	75.0296
PS706K	43.8956	62.4315	70.7374
Bax	43.9159	40.5521	50.1579
Raptor	53.5153	62.4335	68.1684

Table 4. Scalar curvature results for several possible targets in Ewing Sarcoma when samples were treated with MK8669, which is known to inhibit mTOR.

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